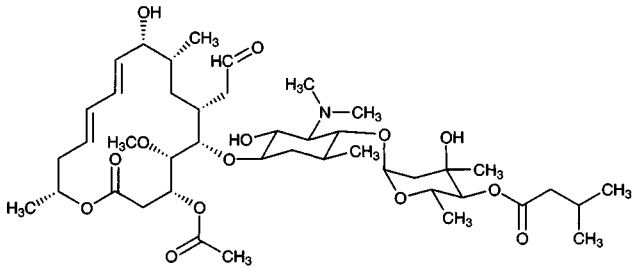
Josamycin

Molecular formula: $C_{42}H_{69}NO_{15}$
Molecular weight: 828.01
CAS Registry No.: 16846-24-5, 51016-68-3 (propionate)
Merck Index: 5280



SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 231.1

CHROMATOGRAM

Retention time: 16.763

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: bulk

HPLC VARIABLES

Column: 250 × 4.6 8 µm 1000 Å PLRP-S (Polymer Labs., UK)
Mobile phase: MeCN:buffer:water 52:20:28 (Prepare buffer by mixing 200 mM K_3PO_4 with 200 mM K_2HPO_4 to obtain a pH of 10.0.)
Column temperature: 60
Flow rate: 1
Injection volume: 20
Detector: UV 232

CHROMATOGRAM

Retention time: 20 (josamycin propionate)

OTHER SUBSTANCES
Simultaneous: josamycin, leucomycin A4 propionate, josamycin 2',9-dipropionate, josamycin 3",9-dipropionate, platenomycin A1 propionate

REFERENCE
Roets,E.; Lepoudre,X.; Van Rompaey,V.; Velghe,G.; Liu,L.; Hoogmartens,J. Liquid chromatography of josamycin propionate on poly(styrene-divinylbenzene), *J.Chromatogr.A*, **1998**, *812*, 303–308.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 1 mL 100 mg Bond-Elut diol SPE cartridge with 1 mL chloroform (Caution! Chloroform is a carcinogen!). Mix 2 g minced muscle tissue with 800 μL water. Stir, vortex for 1 min at maximum speed, let stand for 15 min. Add 2 mL pH 8 buffer, mix briefly, add 10 mL chloroform. Stir at 100 rpm for 15 min, centrifuge at 4000 g for 10 min, discard the aqueous layer, filter the chloroform layer through glass wool. Add the filtrate to the SPE cartridge, wash with 500 μL chloroform, dry under vacuum, elute with three 200 μL portions of MeOH:100 mM ammonium acetate 50:50, inject a 200 μL aliquot of the eluate. (Buffer was 33.46 g K_2HPO_4 and 1.046 g KH_2PO_4 in 1 L water.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm C18
Column: 125 × 4 5 μm Lichrospher RP18
Mobile phase: Gradient. A was MeCN. B was MeOH. C was 0.1% trifluoroacetic acid in water. A:B:C from 20:20:60 to 25:55:20 in 10 (?) min
Flow rate: 0.5
Injection volume: 200
Detector: MS, HP Model 5989 A, desolvation chamber 60°, source 280° and 300° in negative and positive chemical ionization mode, respectively, with methane as reagent, quadrupole 100°, particle beam nebulizer helium 345 kPa, scan m/z 735.4-828.5 in NCI and 828.5-769.4 in PCI

CHROMATOGRAM
Retention time: 7.2
Limit of detection: 50 μg/kg

OTHER SUBSTANCES
Extracted: erythromycin, spiramycin, tilmicosin, tylosin

KEY WORDS
muscle; cow; SPE

REFERENCE
Delépine,B.; Hurtaud-Pessel,D.; Sanders,P. Multiresidue method for confirmation of macrolide antibiotics in bovine muscle by liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1996**, *79*, 397–404.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Bond Elut SCX SPE cartridge (Varian) with 5 mL MeOH and 10 mL 100 mM pH 4.4 KH_2PO_4 buffer. Homogenize 5 g tissue with 100 mL MeOH: 0.3% metaphosphoric acid 30:70 at high speed for 2 min, filter through 2 mm Hyflo Super-Cel coated on a suction funnel (when filtering liver or kidney add several grams of Hyflo Super-Cel to the homogenized solution before filtration). Evaporate the filtrate to ca. 20 mL under reduced pressure at 45°, add to the SPE cartridge, wash with 10 mL distilled water and 5 mL 100 mM pH 8.9 K_2HPO_4 buffer, elute with 10 mL MeOH, evaporate the eluate to dryness under reduced pressure at 45°, dissolve the residue in 1 mL MeCN:50 mM pH 4.5 NaH_2PO_4 buffer 30:70, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Puresil 5C18 (Waters)
Mobile phase: Gradient. A:B from 60:40 to 0:100 over 16 min. A was buffer. B was MeCN:buffer 40:60 (Buffer was 2.5 g KH_2PO_4 dihydrate and 0.65 mL 85% phosphoric acid dissolved in 1 L distilled water, pH 2.5.)

Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 232 for 9 min, UV 287 for 2 min, UV 232 for 4 min

CHROMATOGRAM
Retention time: 13.73
Limit of detection: 50 ng/g

OTHER SUBSTANCES
Extracted: leucomycin (kitasamycin), mirosamicin, spiramycin, tylosin

KEY WORDS
meat; SPE

REFERENCE
Horie,M.; Saito,K.; Ishii,R.; Yoshida,T.; Haramaki,Y.; Nakazawa,H. Simultaneous determination of five macrolide antibiotics in meat by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *812*, 295–302.

SAMPLE
Matrix: tissue
Sample preparation: Blend (Virtis model 45 with U-shaped blades) 2.5 g tissue with 20 mL MeCN:10 mM pH 6.0 phosphate buffer 65:35 for 10 min, centrifuge at 5° at 8500 g for 5 min. Remove the supernatant and adjust the volume to 25 mL with MeCN:10 mM pH 6.0 phosphate buffer 65:35. Remove a 1.5 mL aliquot and add it to 5 mL isooctane, shake for 10 min, centrifuge at 5° at 3000 g for 5 min, discard the organic layer. Add 500 μL reagent to the aqueous layer, mix, heat at 90° for 2 h, cool, inject a 100 μL aliquot. (Prepare reagent by dissolving 1 g cyclohexa-1,3-dione and 25 g ammonium acetate in 60 mL water and 8 mL concentrated HCl, make up to 100 mL with water. Store at 5°, discard after 1 month.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18 end capped
Column: 125 × 4 5 μm LiChrospher 100 RP-18 end capped
Mobile phase: MeCN:MeOH:10 mmole pH 6.0 phosphate buffer 45:5:50
Column temperature: 45
Flow rate: 1.5
Injection volume: 100
Detector: F ex 375 em 450

CHROMATOGRAM
Retention time: 10.3
Limit of detection: 25 ng/g

OTHER SUBSTANCES
Simultaneous: spiramycin, tylosin
Noninterfering: acetaldehyde, benzaldehyde, erythromycin, formaldehyde

KEY WORDS
pig; muscle; liver; kidney; fat; derivatization

REFERENCE
Leroy,P.; Decolin,D.; Nicolas,A.; Archimbault,P. Determination of josamycin residues in porcine tissues using high-performance liquid chromatography with pre-column derivatization and spectrofluorometric detection, *Analyst*, **1994**, *119*, 2743–2747.